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# Effect of hydroxyethyl starches (HES 200/0.5 and HES 130/0.42) on inflamed renal tubular epithelial cells

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# Effect of hydroxyethyl starches (HES 130/0.42 and HES 200/0.5) on inflamed renal tubular epithelial cells

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## ABSTRACT

**Background:** Acute renal failure is a frequent complication of systemic inflammatory response syndrome (SIRS) and sepsis. Hydroxyethyl starches (HES) are widely used in the treatment of such patients. However, the effect of HES on renal function during sepsis remains controversial. This *in vitro* study has been performed to assess possible effects of HES 130/0.42 and HES 200/0.5 on activated proximal tubular epithelial cells.

**Materials and Methods:** Monolayers of HK-2 cells (a cell line of immortalized human proximal tubular epithelial) were stimulated with tumor necrosis factor alpha (TNF-α) (control with phosphate-buffered saline, PBS) in the presence or absence of HES 130/0.42 or 200/0.5 to mimic a septic condition requiring fluid replacement. After 4, 10, and 18 h of incubation monocyte chemoattractant protein-1 (MCP-1), a key chemoattractant for neutrophils and macrophages, was determined. Colorimetric viability- and cytotoxicity assays as well as experiments with FITC-labeled HES preparations were performed.

**Results:** MCP-1 expression was enhanced by 100% ($p < 0.01$) upon TNF-α exposure. In the presence of 2% and 4% HES 200/0.5 during a stimulation time of 10 h and 18 h, MCP-1 concentration was decreased between 22% and 64% ($p < 0.05$). TNF-α stimulation resulted in a significant decrease of viability by 30% ($p < 0.01$), while viability decreased by only 16% in co-incubation with HES 130/0.42 ($p < 0.05$), and remained even unaffected in the presence of HES 200/0.5 ($p < 0.01$). Cell death rate of TNF-α-exposed cells increased by 9%. In

co-incubation with HES 130/0.42 or HES 200/0.5 cell death rate increased by only 1% ($p < 0.01$) and 4% ($p < 0.01$), respectively.

**Discussion and Conclusion:** Data of this *in vitro* study show that in the presence of both HES products an attenuation of cell injury is measured in proximal tubular epithelial cells upon inflammatory stimulation. If these findings are of beneficial or proinflammatory character has further to be investigated.

## INTRODUCTION

Systemic inflammatory response syndrome (SIRS) and sepsis with multiple organ failure remain leading causes of death in intensive care units, despite substantial research in this field over several decades [1]. Pathophysiological changes in patients with SIRS or sepsis are characterized by enhanced expression of inflammatory mediators, accumulation of neutrophils and increased vascular permeability with capillary leakage, which results in interstitial edema formation, decreased intravascular volume and poor perfusion of organs. Adequate fluid management is therefore a key issue in these patients. Clinically, colloids are frequently used for volume replacement when attempting to maintain or improve tissue perfusion in patients experiencing infection, sepsis, trauma, shock, or surgical stress [2-4]. Compared to cristalloids colloids have the advantage of augmenting colloid oncotic pressure and minimizing edema formation, therefore improving oxygen delivery and organ function [5].

Hydroxyethyl starches (HES) are among the most widely used compounds because their volume-expanding effect is both large and long-lasting. There has been extensive research on general effectiveness and safety of colloid administration in septic patients [2,6]. With a special focus on the kidneys both deleterious and protective effects of HES compounds are known [7]. These experiences, however, are based on clinical observations and not on basic research data [8].

Aim of this study was the evaluation of the effect of HES 130/0.42 and HES 200/0.5 on injured human tubular epithelial cells, possibly representing the situation in a septic patient receiving fluid resuscitation with HES. Inflammatory response (production of monocyte chemoattractant protein-1, MCP-1), cell viability and colloid-induced cell death was determined. Additionally, fluorescence labeled-HES was localized in injured cells. We hypothesized that both HES products have proinflammatory characteristics on already injured tubular epithelial cells. Differences regarding effects of these two preparations are not expected.

## MATERIAL AND METHODS

### Cell Culture

HK-2 cells, a human proximal tubule cell line, were used [9]. Cells were cultivated under standard conditions, including temperature (37°C), $CO_2$ concentration (5%), and humidity (95%).

Cultivation was performed in 24-well plates (Corning Inc., NY, USA), in 96-well cell plates (Nunclon™Surface, NUNC, Wiesbaden, Germany), or in 96-strip well plates (Costar®, Corning Inc.). Dulbecco's Modified Eagle Medium (DMEM, Invitrogen, Basel, Switzerland), with 10% fetal bovine serum (FBS, Invitrogen, Basel, Switzerland), 1% Hepes 1 M (Life Technologies, Basel, Switzerland), 1% Penicillin-Streptomycin (Life Technologies, Basel, Switzerland) and 0.1 mg epidermal growth factor (Invitrogen, Basel Switzerland) was used as medium for cultivation.

Cells were used with a 90% confluence. 12 h before experiments were started medium was changed to DMEM/1% FBS with 1% sodium pyruvate (Invitrogen, Basel, Switzerland).

### Experimental Design

Inflammation of HK-2 cells was induced by recombinant tumor necrosis factor alpha (recombinant human TNF-α, BD Pharmingen, Basel, Switzerland) concentrated between 0.1 and 10 ng/ml. HES 130/0.42 and HES 200/0.5 were

provided by BBraun, Switzerland. Experiments were performed in four different groups: control (co), HES, TNF-α (TNF), TNF/HES.

After 2 h of "pre-stimulation" medium of control cells (Co) was changed, while HES cells (HES) were given new medium with HES. TNF-α stimulation was renewed as well after an initial stimulation of 2 h (TNF) or, in the TNF/HES group medium containing TNF-α and HES was replaced. Supernatants and/or cells were collected after 4, 10 or 18 h of stimulation, and cytotoxicity assays and/or viability tests were performed. Supernatants were aliquoted and frozen at -20°C.

**ELISA (Enzyme-linked immunosorbent assay)**

MCP-1 released from HK-2 cells into culture media were determined by enzyme-linked immunosorbent assay (ELISA). Sandwich ELISA was performed according to the manufacturer's protocol assessing human MCP-1 (R&D Systems Europe Ltd.). The detection range for MCP-1 was 0 - 500 pg/ml.

**Cell viability assay**

The MTT-assay is a well-known and acknowledged method to measure cell viability *in vitro* [10]. The method is based on the reduction of the yellow tetrazoliumsalt 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide (MTT) into purple formazan crystals by mitochondrial dehydrogenases. Dehydrogenases are active in living cells only. Conversion of MTT is therefore directly related to cell viability.

MTT was purchased from Sigma (Buchs, Switzerland). A stock solution was prepared diluting 5 mg of MTT in 1 ml PBS. MTT stock solution was added 4 h before end of each time course. Plates were incubated for 4 h at 37°C and supernatants were removed thereafter. Reduction of MTT was stopped by adding MTT solubilization solution from Sigma (Buchs, Switzerland). Data were collected spectrophotometrically using a standard 96-well plate reader (Labsystems Multiskan RC-Elisa reader, BioConcept, Allschwil, Switzerland, wavelength of 570nm).

**LDH-Assay**

Cell death rate (cytotoxicity) was determined using a nonradioactive standard lactate dehydrogenase (LDH) assay (Promega, Madison, WI, USA). We used the LDH assay to measure membrane integrity of the HK-2 cells by quantitatively measuring lactate dehydrogenase (LDH), a stable cytosolic enzyme, that is released upon cell lysis.

Cells were cultivated in 96-well-plates. At the end of each time course cells of some wells of each group were lysed with 10 µl 1% Triton X-100 (Sigma®) to determine total LDH of the cells. 50 µl of supernatants of each well were transferred to a new 96-well plate. 50 µl of the LDH substrate reagent were added to each well already containing 50 µl of the transferred supernatant. The plate was incubated for 30 min a room temperature and kept away from light. Reaction was stopped by 50 µl of 1 M acetic acid. Absorbance was read at a wavelength of 492 nm.

**Labeling of HES with Fluorescein Isothiocyanat (FITC)**

Preperations were made following a slightly modified method as described by DeBelder and Granath (1973): 1.19 g HES (dried at 70°C over $P_2O_5$ in oil pump vacuum) was added to 15 ml dimethylsulfoxide (DMSO, Sigma, Switzerland) and heated at 95°C. After HES was fully dissolved 125 mg fluorescein isothiocyanate (FITC, Fluka, Switzerland) was added. After 6 h the solution was cooled to room temperature and added to 100 ml ethanol, to precipitate the labeled HES. To eliminate any free unreacted FITC, the precipitated HES was dissolved in 20 ml deionized water and dialysed against water for five days. The exclusion volume of the membrane (Spectrum Laboratories, Inc., Ca, USA) was 3.5 g/mol**.** Final isolation of the labeled HES was done by lyophilisation. FITC-labeled HES was then resuspended in 0.9% NaCl to obtain the original concentration of 6%.

**Cellular HES Uptake**

HK-2 cells were cultured on LabTek chamber slides as described above. Inflammation was induced by stimulation with TNF-α (10 ng/ml). After 2 h medium was changed and experimental groups were set up as described for 18 h with 4% FITC-HES. Cells were washed with PBS, fixed with 3% paraformaldehyde in PBS for 2 min, washed again and a 4,6-diamidino-2-phenylindole-staining (DAPI) was performed. After a final wash step, cover slips were mounted by using Dako-glycerol containing 2.5%-diazybicyclo-(2.2.2)-octane (Sigma-Aldrich). Conventional fluorescent images were taken by using an

inverted microscope (Eclipse TE 300/200; Nikon, Germany) equipped with a 60x oil immersion objective, confocal images were taken by a Leica TCS SP 5 UV CLSM (Wetzlar, Germany) laser scan microscope equipped with a 63x oil immersion objective.

**Statistics**

All experiments were performed at least five times with 5 data points for each group. Each data point in the graphs represents mean +/- standard error of the mean (SEM). Analysis of variance (ANOVA) was performed to assess the statistical significance of differences. Differences were considered significant when $p < 0.05$.

## RESULTS

### MCP-1-Expression

*HES 130/0.42*

To evaluate expression of MCP-1 upon stimulation, HK-2 cells were incubated with recombinant TNF-α (control with PBS). After 2 h of stimulation cells were co-incubated with 1% HES 130/0.42 or PBS. Incubation with 1% HES 130/0.42 did not alter MCP-1 expression. MCP-1 significantly increased from 14±1.6 pg/ml to 28±1.8 pg/ml upon TNF-α stimulation ($p< 0.05$) (**Fig. 1A**). Treatment with 1% HES 130/0.42 in the presence of TNF-α did not change MCP-1 expression. Increasing HES 130/0.42 concentrations to 2% and 4% did not influence MCP-1 expression irrespective of the presence or absence of TNF-α (**Fig. 1A**).

Similar data were found, stimulating HK-2 cells with TNF-α for 10 h, adding HES 130/0.42 2 h after initiating TNF-α incubation. While MCP-1 concentration was changed from 16±1.3 pg/ml to 35±3.5 pg/ml upon TNF-α stimulation ($p< 0.05$), 1%, 2%, and 4% HES 130/0.42 did not have any impact on MCP-1 production in the presence of TNF-α (**Fig. 1B**).

18 h stimulation with TNF-α increased MCP-1 concentration from 14±1.2 pg/ml to 28±1.0 pg/ml ($p< 0.01$), while 1%, 2%, and 4% HES 130/0.42 did not alter TNF-α-stimulated MCP-1 expression (**Fig. 1C**).

To evaluate the exact impact of TNF-α stimulation, we altered TNF-α concentrations from 0.1 to 1, 5, and 10 ng/ml TNF-α. Co-incubation with 4% HES 130/0.42 and TNF-α in different concentrations, however, did not lead to

significant changes of MCP-1 expression in comparison to the TNF-α group (**Fig. 2A**).

*HES 200/0.5*

The effect of HES 130/0.42 was compared with HES 200/0.5. The same experiments as mentioned above were performed with HES 200/0.5.

Again, MCP-1 significantly increased from 4±1.5 pg/ml to 33±3.8 pg/ml upon TNF-α stimulation ($p < 0.05$) (**Fig. 3A**). Interestingly, while a 4 h co-incubation with 1% and 2% HES 200/0.5 did not alter expression of MCP-1 in the presence of TNF-α, 4% HES 200/0.5 significantly decreased MCP-1 expression from 29±2.7 pg/ml to 17±3.2 pg/ml ($p < 0.05$) (**Fig. 3A**).

The 10 h time course showed the following change of MCP-1 expression: while 1% HES 200/0.5 did not influence TNF-α-induced production of MCP-1, 2% and 4% decreased MCP-1 from 33±3.5 pg/ml to 20±1.2 pg/ml ($p < 0.05$) and from 31±1.3 pg/ml to 25±0.6 pg/ml ($p < 0.05$), respectively (**Fig. 3B**).

The 18 h TNF-α stimulation with 1% HES 200/0.5 did not decrease MCP - 1 expression as compared with the TNF-α exposure alone, but with 2% HES 200/0.5 MCP-1 expression decreased from 25±0.9 pg/ml to 17±2.5 pg/ml ($p < 0.05$), and with 4% HES 200/0.5 from 29±3.0 pg/ml to 10±1.1 pg/ml ($p < 0.05$) (**Fig. 3C**).

TNF-α concentrations of 0.1 ng/ml, 1 ng/ml, 5 ng/ml, and 10 ng/ml were used for stimulations for 18 h. While TNF-α concentrations of 0.1 and 1 ng/ml in co-incubation with HES 130/0.42 did not show any differences in MCP-1

expression compared to TNF-α alone, TNF-α concentrations of 5 or 10 ng/ml in co-incubation with HES 200/0.5 induced less MCP-1 expression than with TNF-α alone (5 ng/ml TNF-α: 25±2.6 pg/ml vs. 14±0.9 pg/ml ($p< 0.05$); 10 ng/ml TNF-α: 29±3.2 pg/ml vs. 14±2.2 pg/ml ($p< 0.05$) (**Fig. 2B**).

## Determination of Cell Viability

*HES 130/0.42*

Another goal of this study was to evaluate cell viability upon TNF-α stimulation with and without co-incubation with 4% HES 130/0.42. A TNF-α stimulation of 18 h was chosen with varying TNF-α concentrations between 0.1, 1, 5, and 10 ng/ml. Viability decreased by 22% upon stimulation with 0.1 ng/ml TNF-α ($p< 0.05$) (**Fig. 4A**). In the presence of 4% HES 130/0.42, viability decreased only by 7% ($p< 0.05$). With 1 ng/ml TNF-α stimulation, viability decreased by 22% ($p< 0.05$), in the presence of 4% HES 130/0.42, however, only 3% ($p< 0.05$). With 5 ng/ml TNF-α stimulation, viability decreased by 25% ($p< 0.05$) and by 7% ($p< 0.05$) in the presence of 4% HES 130/0.42. With 10 ng/ml TNF-α stimulation finally, viability decreased by 26% ($p< 0.05$) and by 15% ($p< 0.05$) in the presence of 4% HES 130/0.42 (**Fig. 4A**).

*HES 200/0.5*

Experiments were repeated, co-incubating HK-2 cells with 4% HES 200/0.5 in the presence of 0.1, 1, 5, and 10 ng/ml TNF-α. TNF-α- induced decrease of cell viability was 20% upon stimulation with 0.1 ng/ml ($p< 0.05$), 25% with 1 ng/ml ($p<$

0.05), and 36% with 5 ng/ml ($p < 0.05$). Upon stimulation with 10 ng/ml cell viability was impaired by 37 % ($p < 0.05$). A stimulation with 0.1 ng/ml TNF-α in co-incubation with HES 200/0.5 decreased viability by only 10% ($p < 0.05$), with 1 ng/ml viability remained unchanged in comparison to control cells ($p < 0.05$), with 5 ng/ml by 16% ($p < 0.05$), and with TNF-α 10 ng/ml again no change of viability was observed compared to control cells ($p < 0.05$) (p values in comparison to TNF-α stimulation) (**Fig. 4B**).

**Determination of Cytotoxicity**

*HES 130/0.42*

The same experiments were repeated and cytotoxicity was determined. While 0.1 ng/ml TNF-α stimulation did not increase HK-2 cell toxicity compared to PBS-exposed cells, 1, 5, and 10 ng/ml induced cytotoxicity, which was significantly higher (6%, 5%, and 7%, respectively, $p < 0.05$) in comparison to control cells (**Fig. 5A**). Co-incubation with HES 130/0.42 led to no increase of cytotoxicity in the presence of 5 ng/ml TNF-α ($p < 0.05$) and to an attenuation of cytotoxicity of 4% in the presence of 10 ng/ml TNF-α ($p < 0.05$) (p values in comparison to TNF-α stimulation).

*HES 200/0.5*

Incubating HK-2 cells with different concentrations of TNF-α and co-incubating them with 4% HES 200/0.5 gave similar results as with HES 130/0.42. HES 200/0.5 led to an attenuated increase of cytotoxicity: 1% in the presence of 1

ng/ml TNF-α, 2% with 5 ng/ml TNF-α, and 4% with 10 ng/ml TNF-α ($p < 0.05$) (p values in comparison to TNF-α stimulation) (**Fig. 5B**).

**Cellular Uptake of FITC-labeled HES**

Cellular Uptake of FITC-labeled HES was shown for both HES 130/0.42 and HES 200/0.5. However, a visible difference in the quantity of HES uptake regarding both HES compounds was observed when comparing TNF-α stimulated cells with unstimulated cells (**Fig. 6A-D**). To demonstrate an intracellular uptake confocal microscopy was performed. Three-dimensional reproduction showed a clear intracellular accumulation of HES-FITC (**Fig. 7A,B**).

## DISCUSSION

HES has been shown to be beneficial in several inflammatory contexts *in vitro* and *in vivo* by attenuating chemotaxis of white blood cells through endothelial cells, downregulating inflammatory mediators in blood during sepsis, and improving pulmonary function during endotoxemia [11-13]. Additionally, recent data suggests that in sepsis HES may decrease production of hepatic inflammatory mediators [14].

Acute renal failure (ARF) is a common complication of severe sepsis and septic shock [15,16]. Correction of volume depletion is crucial to the prevention of acute tubular necrosis. Data exists that colloids may show advantages in maintaining kidney function in comparison to crystalloid volume replacement [17]. The choice of administered colloid, however, might affect renal function in severely ill patients. Clinical trials pointed out the negative effect of HES 200 on kidney function in sepsis [18,19]. Possible deleterious renal effects of HES have been described even in the absence of an inflammatory situation. In two patients receiving HES 200/0.5 deterioration of pre-existing renal impairment was reported [20]. Two cases of ARF were reported after administration of HES 450/0.7 [21]. Renal insufficiency with biopsy-proven osmotic nephrosis-like lesions was attributed in further cases [22,23]. Evidence has been reported that the use of HES in brain dead organ donors may be associated with osmotic-like lesions of both the proximal and the distal renal tubules [24,25]. These data are supported by another study including 69 kidney transplants from brain-dead donors, which

showed that the need of hemodialysis or hemodiafiltration was significantly higher in the group receiving HES 200/0.62 plus gelatin than in the gelatin group [24]. In another controlled study, urinary output of 24 renal transplant patients was lower in the group receiving HES than no HES [26].

No data exist so far evaluating the impact of HES products on inflamed tubular epithelial cells *in vitro*. We therefore evaluated some aspects of the inflammatory response of human tubular epithelial cells upon stimulation with TNF-α and the co-incubation with HES 130/0.42 or 200/0.5.

In a first experimental setup the inflammatory response was evaluated regarding expression of MCP-1. This chemokine was primarily chosen as the main molecular target, playing a pivotal role not only in monocyte but also in polymorphonuclear cell (PMN) recruitment in various experimental systems [27-31]. Interestingly, HES 130/0.42 did not have an impact on MCP-1 expression in combination with TNF-α stimulation. HES 200/0.5, however, attenuated TNF-α - induced MCP-1 production. This observation points towards a certain difference between HES 130/0.42 and HES 200/0.5 regarding interference with the inflammatory cascade. If decreased expression of MCP-1 is beneficial or harmful has further to be evaluated, but might rather be explained as an attenuation of inflammation. Whether these observed effects are merely due to the fact that HES molecules cover the cell surface and thereby anticipate TNF-α interaction with the receptor, or can be attributed to endocytosis and some yet unknown intracellular target will be an issue of further investigations.

*Previous in vitro* studies on endothelial cell activation suggested a possible beneficial role of HES in the inhibition of endothelial cell activation, preventing neutrophil adhesion upon stimulation with endotoxin  Another study found that HES treatment of PMNs significantly altered tethering to and transmigration through stimulated cultured human endothelial cell monolayers [32]. Results from an *in vitro* study, performed with human microvascular endothelial cells under 2% oxygen for 48 h indicate that hypoxia-induced increases in vascular leakage and acute inflammation are attenuated by HES treatment [33]. Similar results were also achieved in several animals models, showing that HES had an anti-inflammatory effect through suppression of inflammatory mediators. Feng et al. induced sepsis by performing cecal ligation und puncture (CLP) [34]. Animals were randomly assigned to receive saline or HES 130/0.4. Pulmonary inflammation was elucidated. Resuscitation with HES 130/0.4 significantly attenuated the CLP-induced increase of cytokine- and chemokine levels and recruitment of neutrophils. This theory was also confirmed by Lv et al [14]. In the CLP model, HES showed beneficial effects in pulmonary injury through downregulation of inflammatory mediators and suppression of NF-kappaB.

Beside the inflammatory response we were also interested in the effect of both HES products on cell viability. Increased viability of tubular epithelial cells in the presence of HES 130/0.42 and 200/0.5 upon stimulation with TNF-α was observed. Both HES preparations restored the mitochondrial activity, which was impaired by exposure to TNF-α. This is an interesting observation and was not hypothesized. The mechanism of HES-induced cell protection is not clear and

has further to be investigated. Similar observations were made focusing on cell death. While incubation with TNF-α showed a minor cytotoxicity, measured by the release of LDH upon rupture of the cell membrane, HES 130/0.42 as well as HES 200/0.5 prevented it. Also in this case, no difference between HES 130/0.42 and HES 200/0.5 was observed. Interestingly, these data underline a certain HES-induced protection.

Another important goal of this study was to evaluate if tubular epithelial cells ingest HES as observed in other cells such as monocytes, keratinocytes, or perivascular histiocytes [35,36]**.** Experiments with FITC-labeled HES preparations were performed. They indicate an increased cellular uptake of FITC-HES by TNF-α stimulated cells in comparison to unstimulated cells. These data would support the theory, that HES, once intracellular accumulated, could interfere with intracellular pathways.

In summary, our *in vitro* findings of HES-exposed TNF-$\alpha$-injured tubular epithelial cells suggest that the application of HES has potential to attenuate the degree of injury. Well-designed *in vivo* studies, followed by more clinical studies are needed to transfer these results of basic research into clinical practice.

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## FIGURE LEGEND

**Fig. 1A:**

HES 130/0.42 1%, 2%, or 4% administration and expression of monocyte chemoattractant protein-1 (MCP-1). Confluent cell layers were pre-treated with TNF-α 10 ng/ml (or PBS for control) for 2 h, followed by a co-incubation of cells with HES 130/0.42 1%, 2%, or 4% with or without TNF-α 10 ng/ml for 4 h. MCP-1 ELISA was performed with supernatants. Values are mean ± SEM from 5 experiments.

**Fig. 1B:**

HES 130/0.42 1%, 2%, or 4% administration and expression of monocyte chemoattractant protein-1 (MCP-1). Confluent cell layers were pre-treated with TNF-α 10 ng/ml (or PBS for control) for 2 h, followed by a co-incubation of cells with HES 130/0.42 1%, 2%, or 4% with or without TNF-α 10 ng/ml for 10 h. MCP-1 ELISA was performed with supernatants. Values are mean ± SEM from 5 experiments.

**Fig. 1C:**

HES 130/0.42 1%, 2%, or 4% administration and expression of monocyte chemoattractant protein-1 (MCP-1). Confluent cell layers were pre-treated with TNF-α 10 ng/ml (or PBS for control) for 2 h, followed by a co-incubation of cells with HES 130/0.42 1%, 2%, or 4% with or without TNF-α 10 ng/ml for 18 h. MCP-

1 ELISA was performed with supernatants. Values are mean ± SEM from 5 experiments.

**Fig. 2A:**

HES 200/0.5 1%, 2%, or 4% administration and expression of monocyte chemoattractant protein-1 (MCP-1). Confluent cell layers were pre-treated with TNF-α 10 ng/ml (or PBS for control) for 2 h, followed by a co-incubation of cells with HES 200/0.5 1%, 2%, or 4% with or without TNF-α 10 ng/ml for 4 h. MCP-1 ELISA was performed with supernatants. Values are mean ± SEM from 5 experiments.

$P < 0.05$ between TNF and TNF/HES group.

**Fig. 2B:**

HES 200/0.5 1%, 2%, or 4% administration and expression of monocyte chemoattractant protein-1 (MCP-1). Confluent cell layers were pre-treated with TNF-α 10 ng/ml (or PBS for control) for 2 h, followed by a co-incubation of cells with HES 200/0.5 1%, 2%, or 4% with or without TNF-α 10 ng/ml for 10 h. MCP-1 ELISA was performed with supernatants. Values are mean ± SEM from 5 experiments.

$P < 0.05$ between TNF and TNF/HES group.

**Fig. 2C:**

HES 200/0.5 1%, 2%, or 4% administration and expression of monocyte chemoattractant protein-1 (MCP-1). Confluent cell layers were pre-treated with

TNF-α 10 ng/ml (or PBS for control) for 2 h, followed by a co-incubation of cells with HES 200/0.5 1%, 2%, or 4% with or without TNF-α 10 ng/ml for 18 h. MCP-1 ELISA was performed with supernatants. Values are mean ± SEM from 5 experiments.

P < 0.05 between TNF and TNF/HES group.

**Fig. 3A:**

HES 130/0.42 4% administration and expression of monocyte chemoattractant protein-1 (MCP-1). Confluent cell layers were pre-treated with TNF-α concentrated 0.1, 1, 5, or 10 ng/ml (or PBS for control) for 2 h, followed by a co-incubation of cells with HES 130/0.42 4% with or without TNF-α 0.1, 1, 5, or 10 ng/ml for 18 h. MCP-1 ELISA was performed with supernatants. Values are mean ± SEM from 5 experiments.

**Fig. 3B:**

HES 200/0.5 4% administration and expression of monocyte chemoattractant protein-1 (MCP-1). Confluent cell layers were pre-treated with TNF-α concentrated 0.1, 1, 5, or 10 ng/ml (or PBS for control) for 2 h, followed by a co-incubation of cells with HES 200/0.5 4% with or without TNF-α 0.1, 1, 5, or 10 ng/ml for 18 h. MCP-1 ELISA was performed with supernatants. Values are mean ± SEM from 5 experiments.

P < 0.05 between TNF and TNF/HES group.

**Fig. 4A:**

HES 130/0.42 4% administration and cell viability. Confluent cell layers were pre-treated with TNF-α concentrated 0.1, 1, 5, or 10 ng/ml (or PBS for control) for 2 h followed by a co-incubation of cells with HES 130/0.42 4% with or without TNF-α 0.1, 1, 5, or 10 ng/ml for 18 h. Values are mean ± SEM from 5 experiments.

$P < 0.05$ between TNFα and HES-TNFα, and between TNFα and HES.

**Fig. 4B:**

HES 200/0.5 4% administration and cell viability. Confluent cell layers were pre-treated with TNF-α concentrated 0.1, 1, 5, or 10 ng/ml (or PBS for control) for 2 h followed by a co-incubation of cells with HES 200/0.5 4% with or without TNF-α 0.1, 1, 5, or 10 ng/ml for 18 h. Values are mean ± SEM from 5 experiments.

$P < 0.05$ between TNF and control group, and between TNF and TNF/HES.

**Fig. 5A:**

HES 130/0.42 4% administration and cell death. Confluent cell layers were pre-treated with TNF-α concentrated 0.1, 1, 5, and 10 ng/ml (or PBS for control) for 2 h, followed by a co-incubation of cells with HES 130/0.42 4% with or without TNF-α 0.1, 1, 5, or 10 ng/ml for 18 h. Values are mean ± SEM from 5 experiments.

$P < 0.05$ between TNF and control group, and between TNF and TNF/HES.

**Fig. 5B:**

HES 200/0.5 4% administration and cell death. Confluent cell layers were pre-treated with TNF-α concentrated 0.1, 1, 5, and 10 ng/ml (or PBS for control) for 2 h, followed by a co-incubation of cells with HES 200/0.5 4% with or without TNF-α 0.1, 1, 5, or 10 ng/ml for 18 h. Values are mean ± SEM from 5 experiments. $P < 0.05$ between TNF and control group, and between TNF and TNF/HES.

**Fig. 6A-D:**

**A.** Stimulation of HK-2 cells with phosphate-buffered saline for 2 h, followed by exposure to 4% HES-FITC 130/0.42 (green) for 18 h. **B.** Exposure of HK-2 cells to TNF-$\alpha$ for 2 h, followed by a stimulation with TNF-$\alpha$ for 18 h in the presence of 4% HES-FITC 130/0.42 (green). **C.** Stimulation of HK-2 cells with phosphate-buffered saline for 2 h, followed by exposure to 4% HES-FITC 200/0.5 (green) for 18 h. **D.** Exposure of HK-2 cells to TNF-$\alpha$ for 2 h, followed by a stimulation with TNF-$\alpha$ for 18 h in the presence of 4% HES-FITC 200/0.5 (green). Blue DAPI staining represents nuclei of the cells.

**Fig. 7A,B:**

Confocal microscopy showing cellular uptake of 4% HES-FITC (green).

**A.** Exposure of HK-2 cells to TNF-$\alpha$ for 2 h, followed by a stimulation with TNF-$\alpha$ for 18 h in the presence of 4% HES-FITC 130/0.42 (green). **B.** Exposure of HK-2 cells to TNF-$\alpha$ for 2 h, followed by a stimulation with TNF-$\alpha$ for 18 h in the

presence of 4% HES-FITC 200/0.5 (green). Blue DAPI staining represents nuclei of the cells.

**Fig. 1A,B,C:**

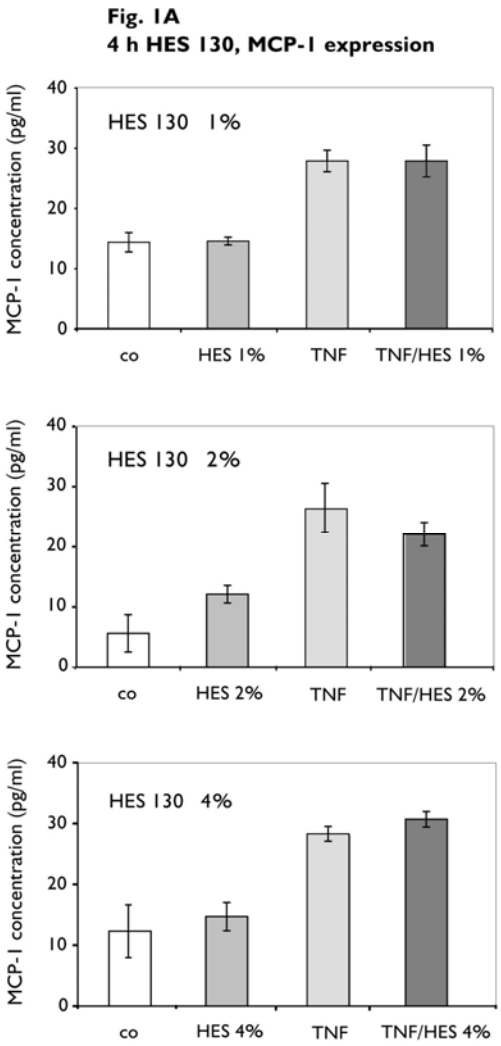


Fig. 1A
4 h HES 130, MCP-1 expression
MCP-1 concentration (pg/ml)
HES 130 1%
co
HES 1%
TNF
TNF/HES 1%
HES 130 2%
HES 2%
TNF/HES 2%
HES 130 4%
HES 4%
TNF/HES 4%

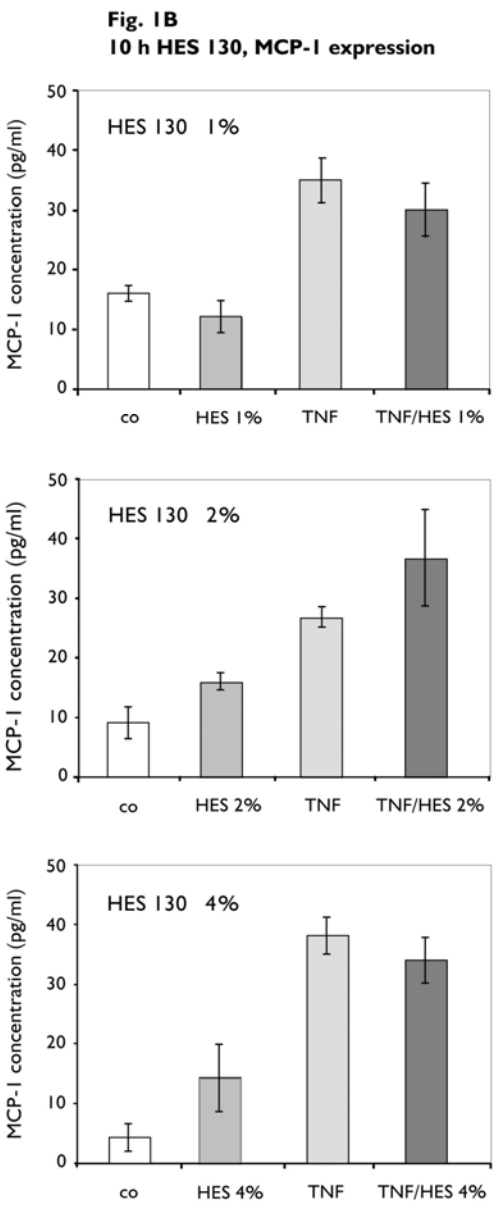


Fig. 1B
10 h HES 130, MCP-1 expression
MCP-1 concentration (pg/ml)
HES 130 1%
co
HES 1%
TNF
TNF/HES 1%
HES 130 2%
HES 2%
TNF/HES 2%
HES 130 4%
HES 4%
TNF/HES 4%

**Fig. 1C**
**18 h HES 130, MCP-1 expression**

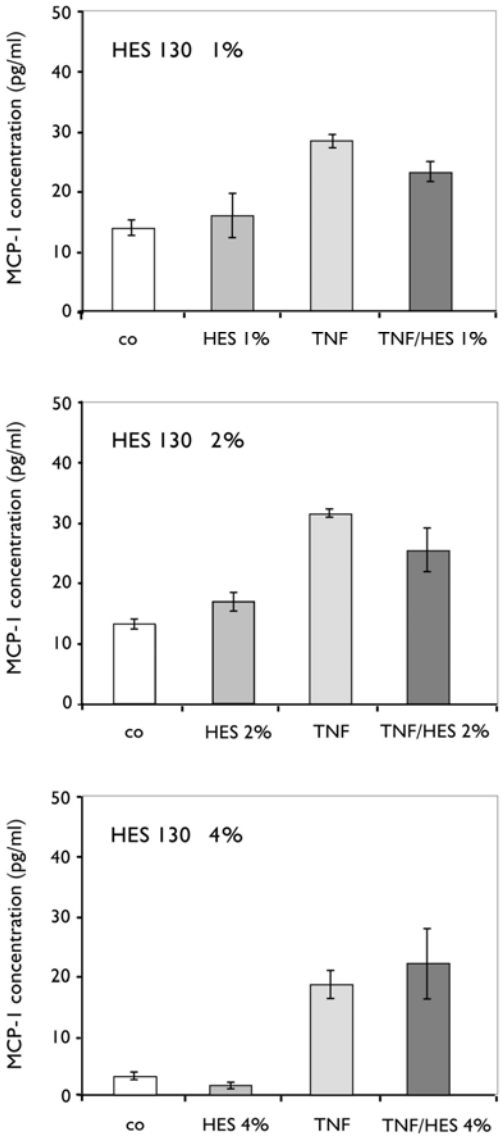


**Fig. 2A,B,C:**

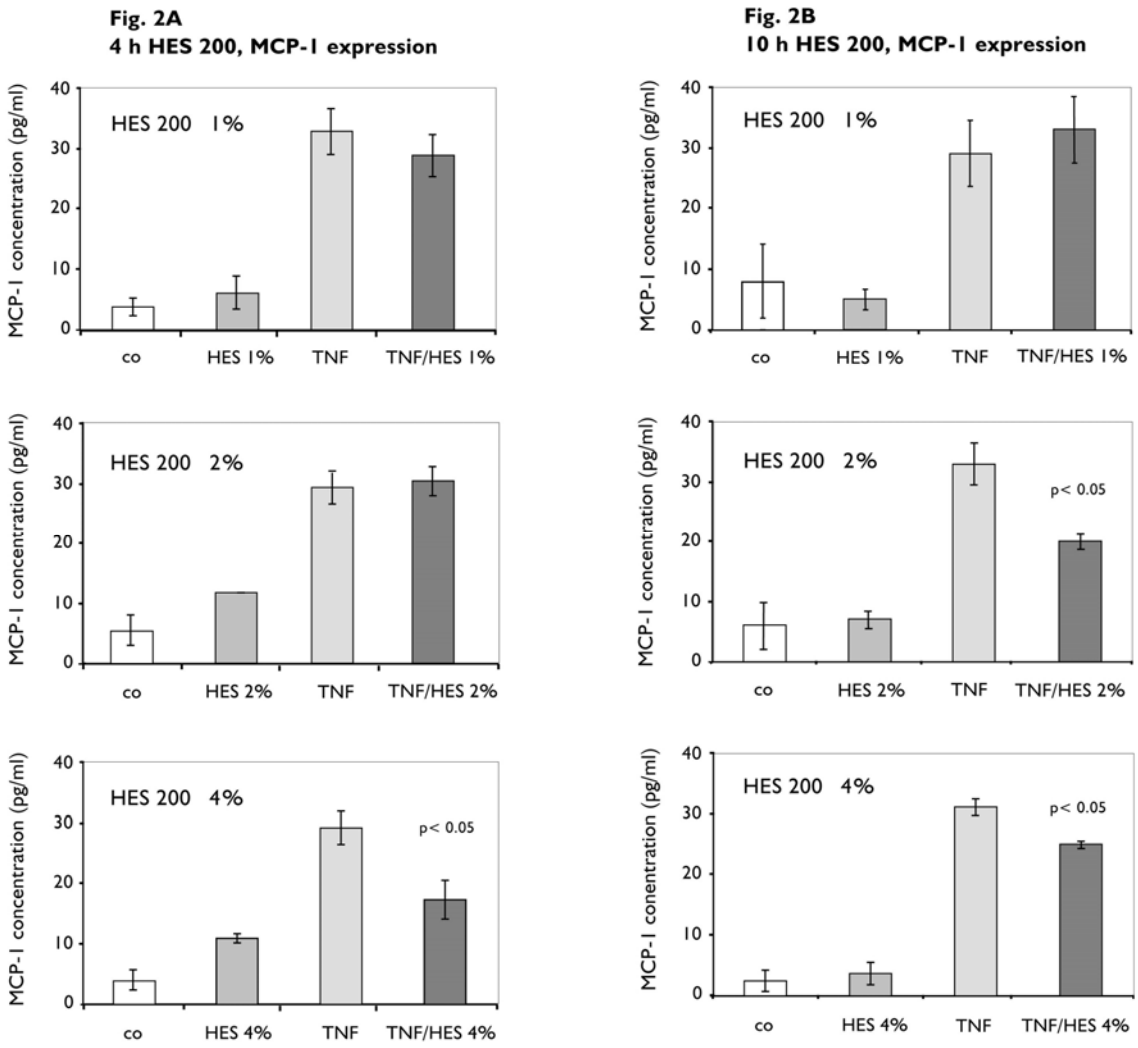


Fig. 2A
4 h HES 200, MCP-1 expression
Fig. 2B
10 h HES 200, MCP-1 expression
MCP-1 concentration (pg/ml)
HES 200 1%
HES 200 2%
HES 200 4%
p< 0.05
co
HES 1%
HES 2%
HES 4%
TNF
TNF/HES 1%
TNF/HES 2%
TNF/HES 4%
MCP-1 conentration (pg/ml)

**Fig. 2C**
**18 h HES 200, MCP-1 expression**

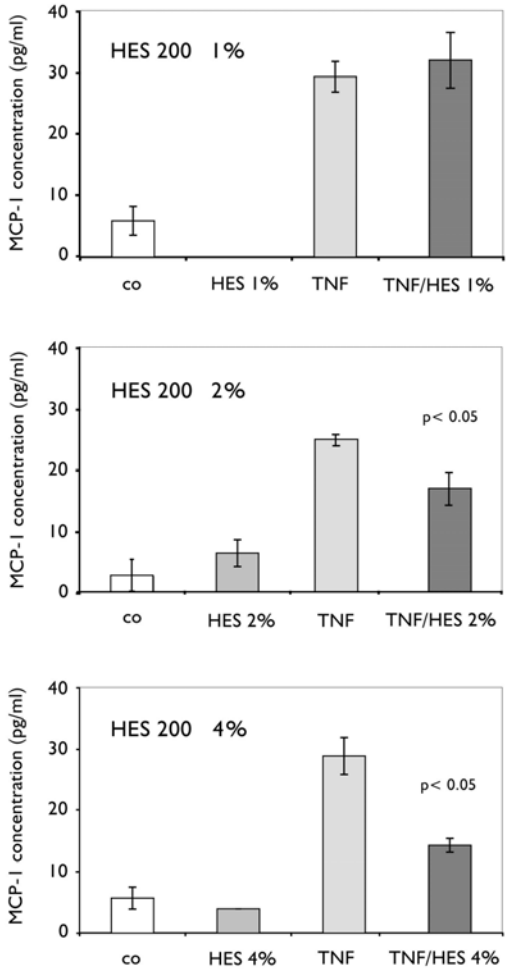


**Fig. 3A,B:**

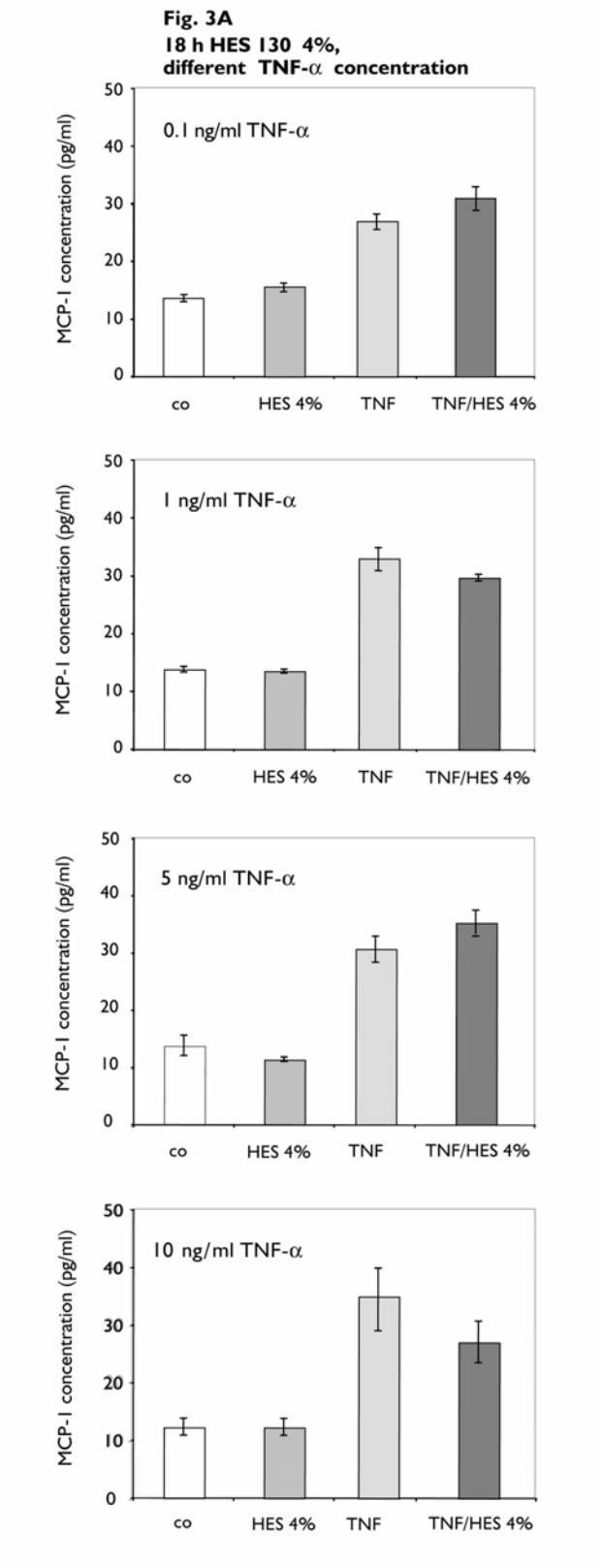


Fig. 3A
18 h HES 130 4%,
different TNF-α concentration
0.1 ng/ml TNF-α
1 ng/ml TNF-α
5 ng/ml TNF-α
10 ng/ml TNF-α
MCP-1 concentration (pg/ml)
0
10
20
30
40
50
co
HES 4%
TNF
TNF/HES 4%

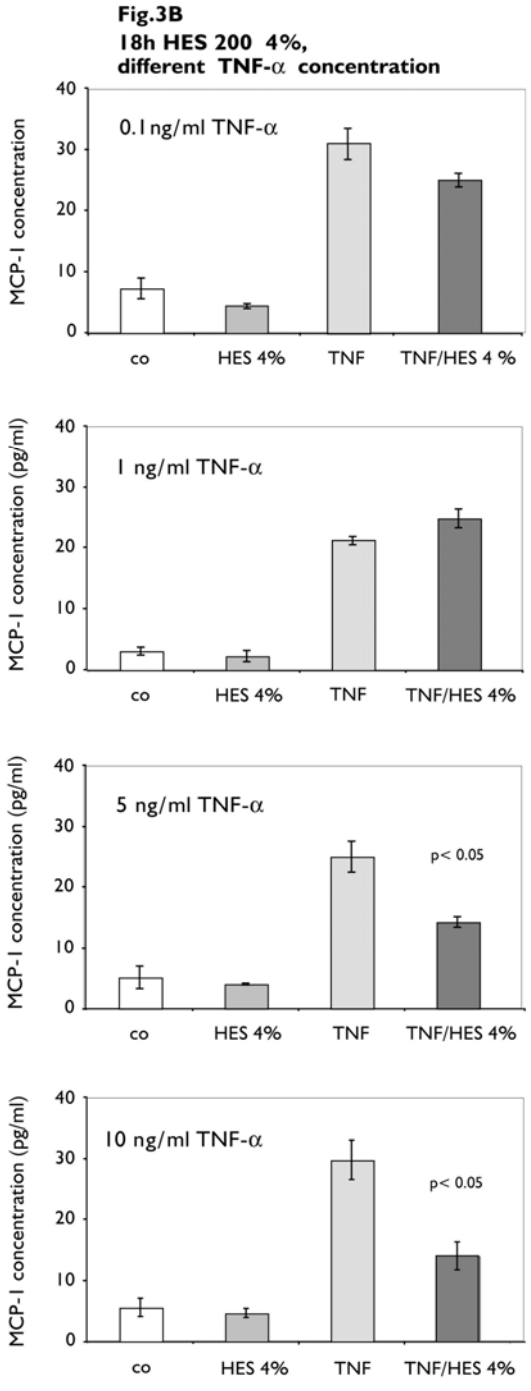


Fig.3B
18h HES 200 4%,
different TNF-α concentration
0.1 ng/ml TNF-α
1 ng/ml TNF-α
5 ng/ml TNF-α
10 ng/ml TNF-α
p< 0.05
p< 0.05
MCP-1 concentration (pg/ml)
0
10
20
30
40
co
HES 4%
TNF
TNF/HES 4 %
TNF/HES 4%

**Fig. 4A,B:**

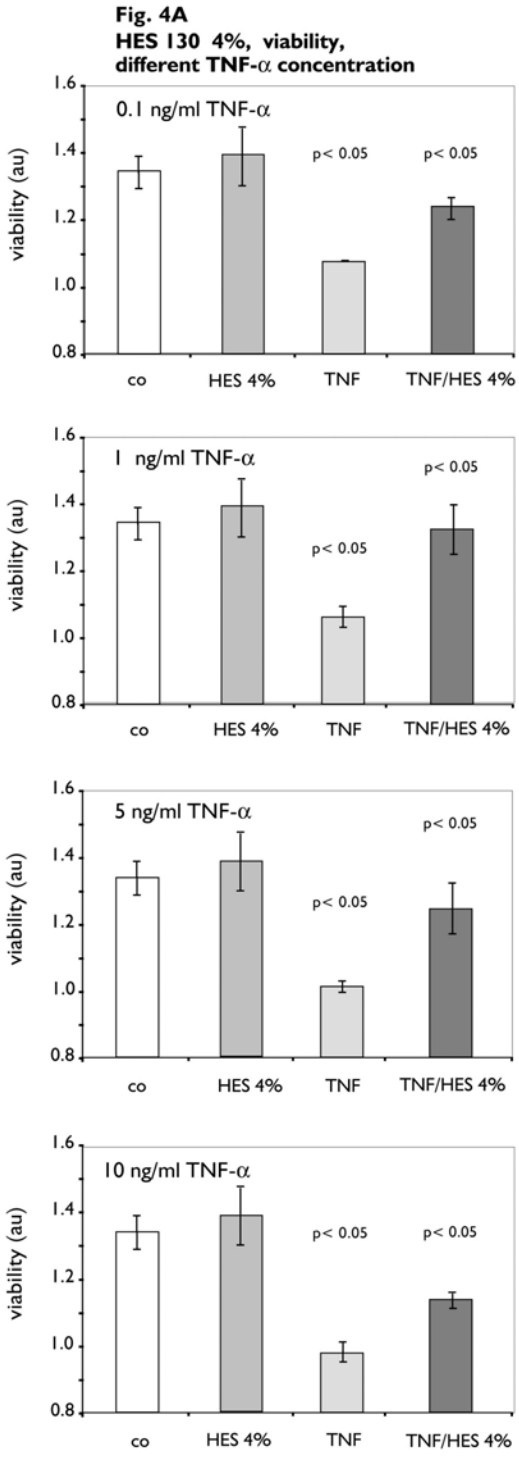


Fig. 4A
HES 130 4%, viability,
different TNF-α concentration
0.1 ng/ml TNF-α
1 ng/ml TNF-α
5 ng/ml TNF-α
10 ng/ml TNF-α
viability (au)
p< 0.05
co
HES 4%
TNF
TNF/HES 4%

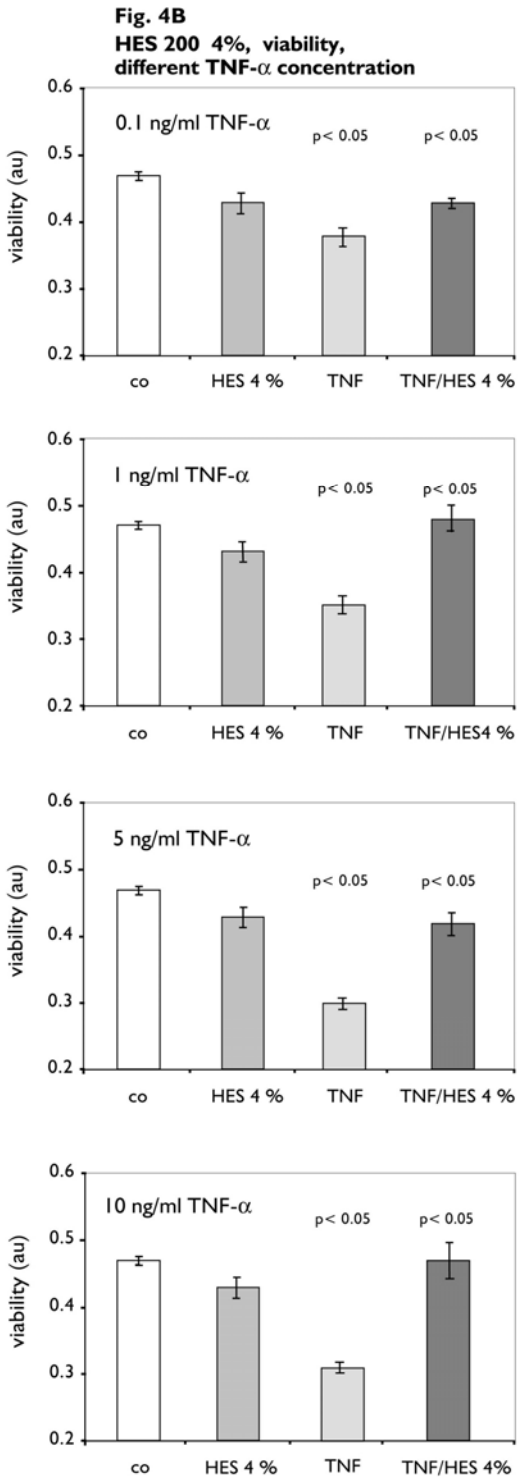


Fig. 4B
HES 200 4%, viability,
different TNF-α concentration
0.1 ng/ml TNF-α
1 ng/ml TNF-α
5 ng/ml TNF-α
10 ng/ml TNF-α
viability (au)
p< 0.05
co
HES 4 %
TNF
TNF/HES 4 %
TNF/HES4 %

**Fig. 5A,B:**

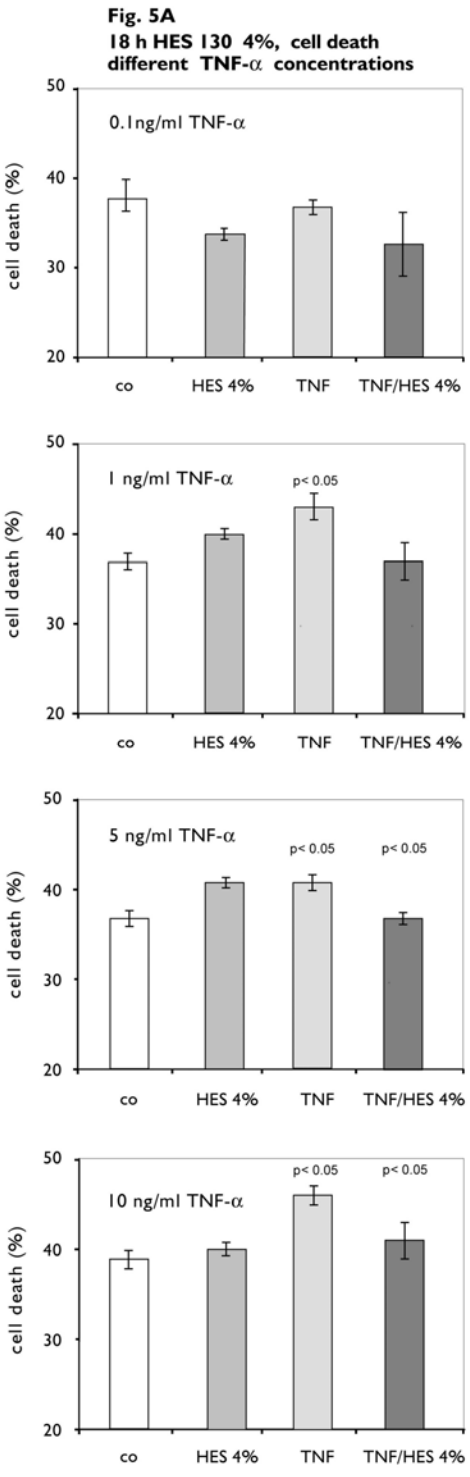


Fig. 5A
18 h HES 130 4%, cell death
different TNF-α concentrations
0.1ng/ml TNF-α
1 ng/ml TNF-α
5 ng/ml TNF-α
10 ng/ml TNF-α
cell death (%)
50
40
30
20
co
HES 4%
TNF
TNF/HES 4%
p< 0.05

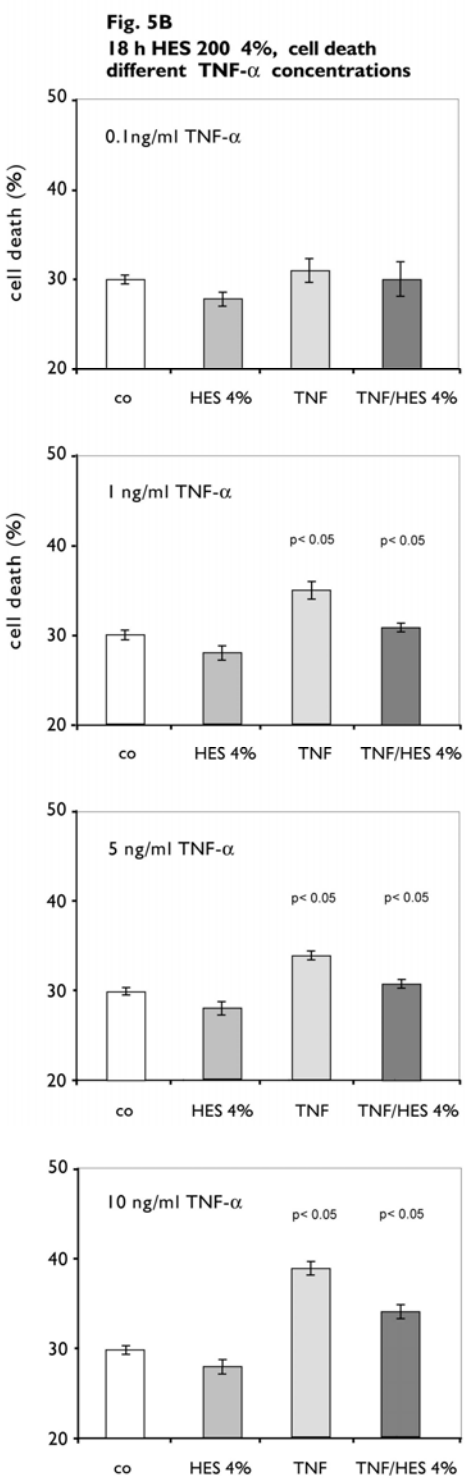


Fig. 5B
18 h HES 200 4%, cell death
different TNF-α concentrations
0.1ng/ml TNF-α
1 ng/ml TNF-α
5 ng/ml TNF-α
10 ng/ml TNF-α
cell death (%)
50
40
30
20
co
HES 4%
TNF
TNF/HES 4%
p< 0.05

**Fig. 6A-D:**

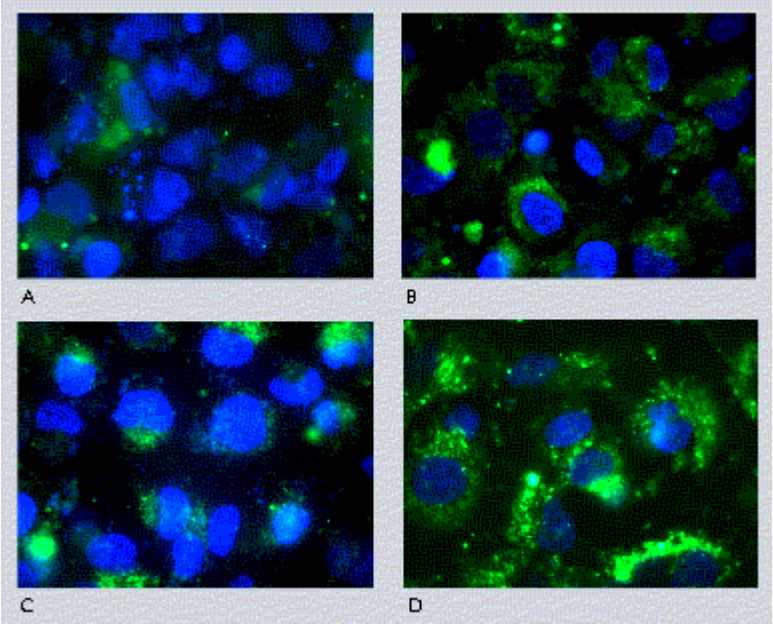
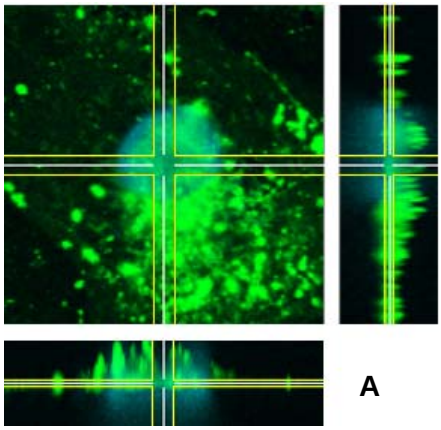
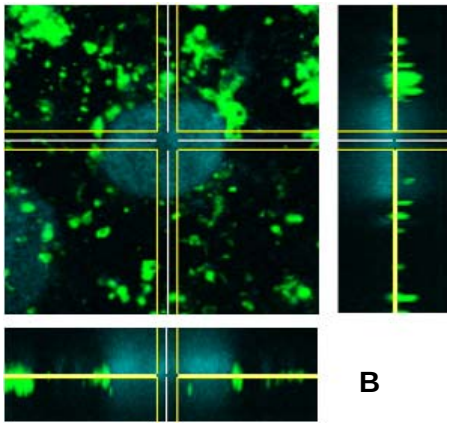



**Fig. 7A,B:**



**A**



**B**

## CURRICULUM VITAE

**Moritz Wittlinger, wohnhaft Schindlerstrasse 15, 8006 Zürich, Schweiz**

| | |
|---|---|
| **23.02.1978** | Geburt als Sohn von Friedrich und Sigrid Wittlinger, geb. Kreis in Münster in Westfalen, Deutschland |
| **1984** | Einschulung in die Grundschule Intrup, Lengerich, Westfalen |
| **1988** | Umschulung in das Hannah-Arendt-Gymnasium Lengerich, Westfalen |
| **Juni 1997** | Abitur |
| **August 1997 - November 1998** | Der andere Dienst im Ausland: Maison Emmanuelle, Quebec, Kanada |
| **Januar 1999 – September 1999** | Ausbildung zum Rettungsassistenten am Matthiasspital in Rheine, Deutschland |
| **Oktober 1999 – September 2000** | Anerkennungsjahr zum staatlich geprüften Rettungsassistenten bei der Feuerwehr Lengerich, Westfalen, Deutschland |
| **Oktober 2000 – September 2002** | Studium der Humanmedizin an der Universität Rostock, Deutschland |
| **September 2002** | Ärztliche Vorprüfung an der Universität Rostock |
| **Oktober 2002 – März 2007** | Studium der Humanmedizin an der RWTH Aachen, Deutschland |
| **August 2003** | Erster Abschnitt der ärztlichen Prüfung an der RWTH Aachen |
| **September 2005** | Zweiter Abschnitt der ärztlichen Prüfung an der RWTH Aachen |
| **November 2006** | Dritter Abschnitt der ärztlichen Prüfung an der RWTH Aachen und Approbation als Arzt |
| **Januar 2007 – Dezember 2007** | Beginn des Doktoratenstudiums am physiologischen Institut der Universität Zürich unter der Leitung von Frau PD Dr. med. Beatrice Beck-Schimmer |
| **Januar 2008** | Assistenzarzt und wissenschaftlicher Assistent am UniversitätsSpital Zürich, Institut für Anästhesiologie unter der Leitung von Prof. Dr. med. Donat R. Spahn |